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3 **1 Inter-individual variation in the adaptive response to heat acclimation**

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5 **2 Running Head:** variability in heat acclimation response

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4 **Original Investigation**

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62 **ABSTRACT**
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65 **Aim:** To investigate inter-individual variance in adaptive responses to heat acclimation (HA).
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67 **Methods:** 17 males ($VO_{2max}=58.8(8.4)$ ml·kg⁻¹·min⁻¹) undertook 10-days (exercise + heat-
68 stress [40°C, 50%RH]) HA. Adaptation was assessed by heat stress tests (HST; 60–minutes
69 cycling, 35% peak power output) pre- and post-HA. **Results:** Inter-individual variability was
70 evident in adaptive responses e.g. mean(range) reduction in end-exercise $T_{re}=-0.70(-0.20$ to -
71 1.32)°C, but, in the main, the variance in adaptation was unrelated across indices (thermal,
72 sudomotor, cardiovascular, haematological), indicating independence between adaptation
73 indices. Variance in adaptive responses was not correlated with aerobic **capacity**, history of
74 previous HA, or the accrued thermal-dose. Some responses to the initial HST were related to
75 the subsequent adaptations e.g. ΔT_{sk} during the initial HST and the reduction in **the** within
76 HST ΔT_{re} after HA ($r=-0.676$), but responses to the initial HST may also have been influenced
77 by HST design e.g. ΔT_{re} correlated with metabolic heat production ($r=0.609$). Metabolic heat
78 production also correlated with the reduction in the within HST ΔT_{re} after HA ($r=-0.514$).
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80 **Summary:** HA indices are mainly independent; ‘low’, or ‘high’, responders on one **index** do
81 not necessarily demonstrate similar response across other indices. Variance in HA responses
82 was not related to aerobic **capacity**, previous HA, or thermal-dose. Thermo-physiological
83 responses to a HST might identify individuals who will benefit from HA. However, some initial
84 responses are influenced by HST design, which may also affect the scope for demonstrating
85 adaptation. **Conclusion:** Variance in the HA response remains largely unaccounted for and future
86 studies should identify factors contributing to this variance.
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109 **KEYWORDS:** Acclimatization; adaptation; thermal; responders; non-responders
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121 51 **HIGHLIGHTS**
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- 123 52 • Although we demonstrated pronounced inter-participant variance in the adaptive
124 response to heat, this was not explained by factors that have putatively been suggested
125 to influence this response, such as maximal aerobic capacity, previous heat acclimation,
126 or the thermal ‘dose’ accrued during the heat intervention.
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133 56 • Classification of individuals as either ‘low’ or ‘high’ responders to heat may not be
134 appropriate. Acclimation indices appear to be largely independent and individuals
135 demonstrating a pronounced, or blunted, adaptive response on one index of acclimation
136 do not necessarily demonstrate a similar response across other indices.
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142 60 • Some of the thermo-physiological responses to an initial heat stress test undertaken
143 before a programme of heat acclimation were related to the magnitude of subsequent
144 adaptation, suggesting that this type of test may have utility in assessing baseline ‘heat-
145 readiness’, as well as in identifying individuals who will most benefit from heat
146 acclimation.
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153 65 • Some of the initial responses may have been influenced by the heat stress test design,
154 which could also affect the scope for demonstrating adaption, although most of the
155 variance in the adaptive response remained unaccounted for.
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1.0 INTRODUCTION

At the cohort level, the typical heat acclimation (HA) response is well characterised (for recent reviews see: Daanen *et al.*, 2018; Périard *et al.*, 2015; Tyler *et al.*, 2016). Broadly speaking, the heat-adapted phenotype is characterised by hypervolemia (Senay *et al.*, 1976), an increased sudomotor response (Nadel *et al.*, 1974), and reduced heart rate, rectal temperature (T_{re}), and mean body temperature (\bar{T}_b) during exercise at a given external work rate in the heat (Neal *et al.*, 2016b; Rendell *et al.*, 2017). However, whilst there is consistency between studies when the adaptive response to heat is viewed at the cohort level, where individual data are presented considerable heterogeneity is evident. For instance, Senay *et al.* (1976) demonstrated a typical group response for the plasma volume increase to a 10-day HA programme, yet the individual data show the final plasma volume expansion ranged from ~8 to 33%. In a related paper large variations in the reduction in exercise heart rate (~-2 to -32 beats·minute⁻¹) and T_{re} (~-0.3 to -1.2°C) were evident following the same 10-day HA programme (Wyndham *et al.*, 1976). Heterogeneity has also been demonstrated in the sudomotor adaptation (sweating rate) following HA (Mitchell *et al.*, 1976). These observations are consistent with later work by Racinais *et al.* (2012) who also noted high inter-individual variation in the adaptive response to a 6-day heat acclimatization programme (*e.g.* change in (Δ) plasma volume of -10 to +20%) with apparent ‘responders’ and ‘non-responders’; similar findings were also reported by Racinais *et al.* (2014) following a 2-week acclimatization intervention. Although the variability reported by Racinais *et al.*, (2012 and 2014) might be attributable to the greater complexity of natural acclimatization compared to laboratory protocols (Edholm, 1966), recent research using a standard 10-day laboratory HA intervention also demonstrated a broad spectrum of adaptive responses to HA (Neal *et al.*, 2016b; Rendell *et al.*, 2017). Interestingly, it is unknown whether the response profile is consistent across HA indices, that is, whether individuals who have a pronounced, or conversely low, adaptive response for a given index of HA, demonstrate the

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93 same response across the range of HA indices. However, cardiovascular changes can occur in
94 the absence of significant alterations in plasma volume (Garrett *et al.*, 2009; Neal *et al.*, 2016a)
95 and reductions in T_{re} and \bar{T}_b post-HA have also been reported without plasma volume changes
96 (Neal *et al.*, 2016a), whereas the plateau in the T_{re} adaptation during HA may precede
97 pronounced sudomotor adaptation (Périard *et al.*, 2015). Given the apparent independence
98 between some aspects of the HA response, it might be anticipated that the magnitude of
99 response is specific to the HA index.

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101 Understanding the basis for heterogeneity in the HA response has important practical utility
102 for the screening and identification of individuals who will most benefit from undertaking HA,
103 in optimising the HA process, and in identifying those individuals best suited to performing in
104 a hot environment or those at increased risk of an adverse response e.g. poor heat tolerance
105 (Epstein, 1990). The need to increase understanding of the factors underpinning the inter-
106 individual variability in the HA response was highlighted as a priority in a 2012 International
107 Olympic Committee consensus statement (Bergeron *et al.*, 2012), yet little subsequent progress
108 has been made. Historic work suggests that a dose-response relationship between heat exposure
109 and the magnitude of the adaptive response underpins some of the variability in the HA
110 response (Fox *et al.*, 1963; Lind & Bass, 1963) although there may be a ceiling-effect for
111 thermal ‘dose’ given that elevating T_{re} beyond 38.5°C during a HA intervention does not confer
112 any additional benefit (Gibson *et al.*, 2015). Moreover, there is some evidence to suggest that
113 individuals with a high maximal aerobic capacity (VO_{2max}) may be partially heat acclimated
114 (Ravanelli *et al.*, 2018; Shvartz *et al.*, 1977), probably by virtue of some of their training
115 adaption (e.g. hypervolemia) as well as through the high thermal-strain that can be elicited
116 through their habitual exercise at high absolute exercise intensities under temperate conditions
117 (Ely *et al.*, 2009). Similarly, individuals with a high VO_{2max} may acclimate more rapidly than

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298 118 individual with a lower $\text{VO}_{2\text{max}}$ (Pandolf *et al.*, 1977), whereas meta-analytic data suggests that
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300 119 the process of re-acclimation is more rapid than initial HA (Daanen *et al.*, 2018) and animal
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302 120 models have provided evidence of a HA memory, at least in terms of cytoprotection (Horowitz,
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304 121 2016). Finally, whilst the roles of $\text{VO}_{2\text{max}}$ and anthropometric factors have historically been
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306 122 emphasised in explaining the thermophysiological responses to exercise in the heat, recent
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308 123 work has demonstrated that the metabolic heat production (H_{prod}) explained the largest amount
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310 124 of the inter-individual variance in the ΔT_{re} whereas the evaporative requirement for heat
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312 125 balance (E_{req}) explained the largest amount of variance in sweating rate (Cramer and Jay, 2015).
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314 126 However, there has, historically, been no attempt to standardise these parameters in protocols
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316 127 for assessing HA (*e.g.* Garrett *et al.*, 2009; Gibson *et al.*, 2015; Neal *et al.*, 2016ab; Pandolf *et*
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318 128 *al.*, 1977; Rendell *et al.*, 2017; Senay *et al.*, 1976; Shvartz *et al.*, 1977) raising the possibility
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320 129 that the design of the heat stress test (HST) might contribute to the variance observed in the
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322 130 response to the initial baseline assessment of acclimation state. Likewise, it might be
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324 131 anticipated that a large increase in T_{re} during the HST prior to HA, which might be influenced
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326 132 by H_{prod} rather than acclimation state *per se*, could provide the greatest scope for demonstrating
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328 133 an adaptive response thereafter.
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334 135 Accordingly, the primary aim of the present study was to examine the putative factors
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336 136 underpinning the observed variance in the adaptive response to a standard HA intervention.
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338 137 We hypothesised that $\text{VO}_{2\text{max}}$, a history of previous HA, the thermal dosage experience during
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340 138 the HA intervention, and the baseline response to a standard HST), would be significant
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342 139 contributors to the variance in the HA response. We also investigated the extent to which the
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344 140 inter-individual variability in the magnitude of adaptive response to heat was consistent across
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346 141 adaption indices, that is, whether individuals who have a pronounced, or conversely low,
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348 142 adaptive response on one index of HA demonstrate a similar response across other indices of
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357 143 HA, or whether the magnitude of adaptive response is specific to the **index** of adaption. Based
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359 144 upon the apparent independence between some indices of HA we hypothesised that the
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361 145 response profile would be non-uniform. Finally, we investigated the factors influencing the
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363 146 thermo-physiological responses to the initial HST, and whether this influenced the subsequent
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365 147 adaptive response to a HA **intervention**. Our hypothesis was that the highest T_{re} and whole-
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367 148 body sweating rates (WBSR) in the initial HST would be observed in those individuals with
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369 149 the highest H_{prod} and E_{req} , respectively, and that these high baseline responses would provide
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371 150 greater scope for evidencing adaptation.
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376 152 **2.0 METHODS**

378 153 **2.1 Participants**

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381 154 **Seventeen** trained males participated (Mean(SD) age: 22(5) years; height: 1.81(0.05) m; mass:
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383 155 74.4(6.3) kg; body surface area (BSA, Dubois and Dubois, 1916) 1.94(0.10) m²; VO_{2max} :
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385 156 58.8(8.4) mL·kg⁻¹·min⁻¹). These data were pooled from previously published studies (Neal *et*
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387 157 *al.*, 2016b; Rendell *et al.*, 2017). The studies received ethical approval from the Universities
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389 158 Science Faculty ethics committee and were conducted in accordance with the Declaration of
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391 159 Helsinki (2013). All participants completed a health history questionnaire and provided written
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393 160 informed consent.
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398 162 **2.2 Experimental design**

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400 163 A within-participant repeated-measures design was employed. All participants undertook a
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402 164 preliminary graded exercise test (GXT) under temperate ambient conditions (target ambient
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404 165 conditions: 22°C; 50%RH) in the seven day period prior to commencing the HA **intervention**.
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406 166 Thereafter, participants undertook 11 consecutive days of exercise-heat exposures (target
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408 167 ambient conditions: 40°C; 50%RH). The first, sixth and eleventh day consisted of a
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416 168 standardised exercise HST for assessing the HA responses; the other days consisted of exercise-
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418 169 heat exposures using the controlled **hyperthermia** (CH) approach. Nine of the participants had
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421 170 previously undertaken a heat acclimation programme (3 to 18 months washout).
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424 172 **2.3 Experimental procedures**

425 173 **2.3.1 Graded Exercise Test**

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429 174 Participants initially cycled (Excalibur, Lode, The Netherlands) at 85-110 W, dependent upon
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431 175 the estimated fitness of the participant. After 20 minutes work-rate was incremented by 25 W
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433 176 every 3 minutes until fingertip capillary blood lactate concentration [Lac] was ≥ 4 mmol·L⁻¹
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435 177 (Biosen C-line, EKF Diagnostic, Cardiff, UK). Thereafter, following a five-minute recovery
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437 178 period, the participant cycled at 100 W for five minutes, before work-rate was increased by 25
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439 179 W·min⁻¹ until volitional exhaustion. $\text{VO}_{2\text{max}}$ was defined as the highest 15 s VO_2 .
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443 181 **2.3.2 Exercise-Heat Stress Test**

444 182 Participants cycled on a calibrated CompuTrainer cycle ergometer (RacerMate Inc., Seattle,
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446 183 Washington, USA) for 60 minutes at 35% of the GXT peak power. All HSTs were completed
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448 184 at the same time of day, within-participant.
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453 186 **2.3.3 Controlled Hyperthermia**

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455 187 Participants self-selected their initial work rate on the Computrainer cycle ergometer in order
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457 188 to elicit a target rating of perceived exertion (RPE [Borg, 1982]) of 15. This was maintained
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459 189 until $T_{\text{re}}=38.3^\circ\text{C}$, at which point external power output and convective cooling ($\sim 2\text{-}3$ m·s⁻¹)
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461 190 were adjusted as appropriate to maintain the target T_{re} ($38.5\text{-}38.7^\circ\text{C}$). Convective cooling was
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463 191 manipulated to facilitate the exercise component and provide some perceptual benefit, whilst
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465 192 maintaining a high mean skin temperature. The total exercise-heat exposure was 90-minutes
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475 193 per session. The time each individual spent with a $T_{re} > 38.5^{\circ}\text{C}$ during the CH sessions was used
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477 194 as an index of the thermal ‘dose’ accrued during the HA **intervention** as used previously
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479 195 (Zurawlew *et al.*, 2016).
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483 484 197 **2.4 General procedures**

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486 198 Participants wore the same clothes (shorts, undergarments, shoes) each day, abstained from
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488 199 alcohol throughout the experimental period and caffeine for 12 hours before exercise, and were
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490 200 instructed to consume a similar diet before each test and drink 500 mL of water 2 hours before
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492 201 every attendance. Participants were instructed to maintain their normal high-intensity training
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494 202 (except 24 hours before HSTs or GXTs) and replace an equivalent duration of low/moderate
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496 203 training with that completed in the laboratory to maintain usual training volume. To estimate
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498 204 WBSR, nude body mass was measured immediately before and after every exercise session
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500 205 (Industrial Electronic Weight Indicator, Model I10, Ohaus Corporation, Parsippany, New
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502 206 Jersey, USA), having adjusted for fluid consumption. During HST and controlled hyperthermia
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504 207 sessions 250 mL boluses of 3.6% carbohydrate solution (drink temperature 20°C) were
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506 208 ingested, immediately prior to commencing exercise and every 15 minutes thereafter. After
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508 209 every exercise session, participants were encouraged to drink *ad libitum* to ensure similar
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510 210 hydration for each of the following days.
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516 212 Ambient conditions were measured by a **wet-bulb globe temperature** (WBGT) logger (Squirrel
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518 213 1000, Grant Instruments, Cambridge, UK), T_{re} by a thermistor (Grant Instruments, Cambridge,
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520 214 UK) self-inserted approximately 15 cm beyond the anal sphincter and cardiac frequency (f_c) by
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522 215 short range telemetry (Polar RS800, Polar Elector, Kempele, Finland). Participants were
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524 216 withdrawn from a session if $T_{re} > 40^{\circ}\text{C}$. During HSTs and GXTs, skin temperature (T_{sk}) was
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526 217 measured using thermistors on the chest, biceps, thigh and calf (Grant Instruments, Cambridge,
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218 UK). During HSTs expired gases (Douglas bag method) were measured at 15 minute intervals.

219 VO₂ was measured breath-by-breath throughout the GXTs (Quark B2, COSMED, Rome, Italy).

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221 Before and after HSTs 10 mL venous blood samples were obtained from the antecubital vein

222 for the triplicate measurement of haemoglobin concentration [Hb] (201+ HemoCue, Sweden)

223 and haematocrit (Hct) (Hawksley, England). Blood volume changes were determined

224 according to Dill and Costill (1974).

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226 **2.5 Data analysis**

227 Mean skin (T_{sk}) calculated according to Ramanathan (1964) with T_b calculated using a two-

228 compartment model (Jay *et al.*, 2007). H_{prod} was calculated according to ISO 8896 (Malchaire,

229 2004). The rate of dry heat exchange (H_{dry}) was calculated as:

230 $H_{dry} = C + R \text{ (W/m}^2\text{)}$

231 $C = h_c (T_{sk} - T_a) \text{ (W/m}^2\text{)}$

232 $R = h_r (T_{sk} - T_r) \text{ (W/m}^2\text{)}$

233 C and R represent convective and radiant heat exchange, respectively, T_a and T_{sk} denote

234 ambient and mean skin temperatures (°C), respectively, T_r is the mean radiant temperature (°C),

235 assumed to the equivalent to ambient temperature in the laboratory setting, h_c is the convective

236 heat transfer coefficient, and h_r is the radiant heat transfer coefficient:

237 $h_c = 8.3 v^{0.6} \text{ (W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}\text{)}$

238 $h_r = 4\varepsilon\sigma (BSA_r/BSA) ((T_{sk} + T_r) / 2 + 273.15)^3 \text{ (W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}\text{)}$

239 Where: v is air velocity ($\text{m}\cdot\text{s}^{-1}$), ε is skin emissivity (0.95), σ is the Stefan-Boltzmann constant

240 ($5.67\cdot 10^{-8} \text{ W}\cdot\text{m}^{-2}\cdot\text{K}^{-4}$), and BSA_r/BSA is the non-dimensional effective radiant surface area

241 for a seated individual valued at 0.70.

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243 Respiratory heat exchange (H_{resp}) was calculated as:

$$E_{\text{res}} + C_{\text{res}} = 0.0173(H_{\text{prod}})(5.87 - P_a) + 0.0014(H_{\text{prod}})(34 - T_a) \text{ (W/m}^2\text{)}$$

245 Where: E_{res} and C_{res} are evaporative and convective heat loss from the respiratory tract,
246 respectively, and P_a is the ambient vapor pressure (kPa).

248 The rate of evaporation required for heat balance (E_{req}) was expressed as:

$$E_{\text{req}} = H_{\text{prod}} - H_{\text{dry}} - H_{\text{resp}} \text{ (W/m}^2\text{)}$$

251 The maximum rate of evaporation to the environment (E_{max}) was determined by:

$$E_{\text{max}} = h_e (P_{\text{sk,s}} - P_a) \text{ (W/m}^2\text{)}$$

253 where h_e is the evaporative heat transfer coefficient, calculated as the product of h_c and the
254 Lewis relation coefficient (16.5 K/kPa), and $P_{\text{sk,s}} - P_a$ is the skin-air vapor pressure gradient

256 The value of $P_{\text{sk,s}}$ was calculated based on T_{sk} using Antoine's equation:

$$P_{\text{sk,s}} = 10 \cdot \exp [18.956 - 4.030.18/(T_{\text{sk}} + 235)] \text{ (kPa)}$$

259 As per convention, heat balance parameters were calculated in W/m^2 ; however, these values
260 are expressed in W or W/kg^{-1} where appropriate.

262 2.6 Statistical Analysis

263 Heat acclimation was assessed using the data obtained from the pre vs. post HA HSTs
264 conducted on day 1 and day 11 of the HA **intervention**. A range of indices were used to assess
265 HA including: thermal (end-exercise T_{re} and T_{b} , the within HST ΔT_{re} and ΔT_{b}),
266 cardiovascular (average exercise heart rate), sudomotor (WBSR), and haematological (Δ blood
267 volume). Data are expressed as mean(SD) unless otherwise stated. **To identify the factors**

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268 influencing the thermo-physiological responses to the initial HST we used an approach similar
269 to that described by Cramer and Jay (2015). Statistical analyses were undertaken using SPSS
270 (IBM Version. 22, IBM, New York, New York, USA) with alpha set *a priori* as ≤ 0.05 . Strength
271 of relationship between variables was assessed using Pearson's product-moment correlation.

Pre HA	Post HA	Pre-post HA
Mean(SD)	Mean(SD)	change
min:max	min:max	Mean(SD)
		min:max

272 Correlation coefficients were considered as strong (≥ 0.60), moderate (0.40 to 0.59), and weak
273 (0.20 to 0.39) (Cohen, 1998). Within-individuals differences were assessed by paired samples
274 t-test. Between-individuals differences were assessed by independent samples t-tests.

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276 **3.0 RESULTS**

277 At the cohort level, a clear adaptive response was evident following the HA programme, as
278 evidenced by significant reductions in the mean(SD) end-exercise T_{re} and $T_{\square b}$, a reduced
279 within HST ΔT_{re} and $\Delta T_{\square b}$, a lower average exercise heart rate, increased WBSR and
280 pronounced hypervolemia. However, inspection of the individual responses revealed notable
281 inter-participant variation in the range of adaptive responses (Table 1).

End exercise T_{re} (°C)	38.79(0.43) 38.18:39.74	38.09(0.40)*** 36.98:38.71	-0.70 (0.34) -0.20:-1.32
ΔT_{re} (°C)	1.53(0.53) 0.78:2.34	1.23(0.44)** 0.37:2.17	-0.30(0.32) 0.47:-0.71
End Exercise T_{\square_b} (°C)	38.65(0.46) 38.02:39.65	37.86(0.35)*** 37.15:38.62	-0.79(0.29) -0.35:-1.25
ΔT_{\square_b} (°C)^a	1.51(0.45) 0.82:2.16	1.13(0.33)*** 0.50:1.71	-0.38(0.27) 0.27:-0.79
Whole body sweat rate (L·hr⁻¹)	1.45(0.33) 1.09:2.22	1.79(0.49)*** 1.15:2.89	0.34(0.29) 0.02:1.03
Blood volume (%)	100.0(0.0) 100.0:100.0	106.5(2.8)*** 102.0:112.9	6.5(2.8) 2.0:12.9
Average heart rate (beats·minute⁻¹)^a	150(11) 135:174	129(8)*** 120:144	-21(5) -12:-29

Table 1: Effect of heat acclimation (HA) on thermophysiological indices measured during a standard heat stress test undertaken before and after heat acclimation (n=17, except ^a where n=16).

Significant difference from pre HA is denoted by: ** = P < 0.01; *** = P < 0.001.

The inter-participant range for VO_{2max} , expressed in absolute terms, was 3.49 to 5.05 L·min⁻¹. Absolute VO_{2max} was not related to the magnitude of reduction in end-exercise T_{re} (P = 0.930) or T_{\square_b} , (P = 0.785), the reduction in the within HST ΔT_{re} (P = 0.722) or ΔT_{\square_b} (P = 0.714), the increase in WBSR (P = 0.405) or blood volume (P = 0.410) or the reduction in average heart rate (P = 0.086) following the HA intervention. The inter-participant range for VO_{2max} , expressed in relative terms was 45.2 to 74.6 mL·kg⁻¹·min⁻¹. The individual relative VO_{2max} was not related to the magnitude of reduction in end-exercise T_{re} (P = 0.947) or T_{\square_b} (P = 0.686) the reduction in the within HST ΔT_{re} (P = 0.852) or ΔT_{\square_b} (P = 0.868), the increase in WBSR (P =

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770 305 0.252) or blood volume (P = 0.381), or the reduction in average heart rate (P = 0.089) following
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772 306 the HA intervention.
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777 308 Nine of the participants had undergone a prior HA **intervention** before participating in the
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779 309 present study. Independent samples t-test indicated that prior experience of heat acclimation
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781 310 did not affect the reduction in end-exercise T_{re} (prior heat exposure = $-0.32(0.34)^{\circ}\text{C}$ vs. no prior
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783 311 exposure = $-0.40(0.19)^{\circ}\text{C}$, P = 0.555) or T_{\square_b} (prior heat exposure = $-0.83(0.25)^{\circ}\text{C}$ vs. no prior
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785 312 exposure = $-0.74(0.33)^{\circ}\text{C}$, P = 0.566), the reduction in the within HST ΔT_{re} (prior heat
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787 313 exposure = $-0.39(0.22)^{\circ}\text{C}$ vs. no prior exposure = $-0.19(0.39)^{\circ}\text{C}$, P = 0.194) or ΔT_{\square_b} (prior
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789 314 heat exposure = $-0.39(0.19)^{\circ}\text{C}$ vs. no prior exposure = $-0.38(0.36)^{\circ}\text{C}$, P = 0.980), increase in
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791 315 WBSR (prior heat exposure = $0.25(0.18) \text{ L}\cdot\text{hr}^{-1}$ vs. no prior exposure = $0.45(0.36) \text{ L}\cdot\text{hr}^{-1}$, P =
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793 316 0.158) and blood volume (prior heat exposure = $6.9(3.1)\%$ vs. no prior exposure = $6.1(2.6)\%$,
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795 317 P = 0.581) or the reduction in average exercise heart rate (prior heat exposure = $-15(8)$
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797 318 $\text{beats}\cdot\text{min}^{-1}$ vs. no prior exposure = $-11(6) \text{ beats}\cdot\text{min}^{-1}$, P = 0.264).
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802 320 The total time individual spent in CH sessions with a $T_{re} > 38.5^{\circ}\text{C}$ was $456(64)$ minutes (range
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804 321 $326:552$ minutes). Total time spent with a $T_{re} > 38.5^{\circ}\text{C}$ was not significantly correlated with
805
806 322 any of the adaption indices. Likewise, the average external work rate sustained during each CH
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808 323 session, expressed either in absolute ($101(16) \text{ W}$, range $69:130 \text{ W}$) or relative terms
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810 324 ($1.37(0.28) \text{ W}\cdot\text{kg}^{-1}$, range $0.86:1.99 \text{ W}\cdot\text{kg}^{-1}$) was not correlated with the reduction in the end-
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812 325 exercise T_{re} (P = 0.986, P = 0.939, respectively), end-exercise T_{\square_b} (P = 0.489, P = 0.888,
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814 326 respectively), the within session ΔT_{re} (P = 0.614, P = 0.981, respectively), ΔT_{\square_b} (P = 0.718, P
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816 327 = 0.620, respectively), Δ blood volume (P = 0.726, P = 0.344, respectively) and the reduction
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818 328 in average exercise heart rate (P = 0.077, P = 0.068, respectively). However, there was a
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820 329 significant moderate negative relationship between the average absolute power sustained in
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829 330 each CH session and the increase in WBSR ($r = -0.530$, $P = 0.029$), but the relative power
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831 331 sustained in each CH session was not significantly related to WBSR ($P = 0.054$).
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836 333 The baseline responses to the pre-HA HST were correlated with a number of the adaption
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838 334 indices (Figure 1 a-f). The reduction in end-exercise T_{re} following HA was correlated with the
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840 335 pre-HA HST end-exercise T_{re} ($r = -0.490$, $P = 0.046$) and the baseline [Hb] ($r = 0.550$, $P =$
841
842 336 0.022). The reduction in the within session ΔT_{re} following HA was correlated with the end
843
844 337 exercise T_{sk} ($r = -0.529$, $P = 0.029$), ΔT_{sk} ($r = -0.676$, $P = 0.004$) and ΔT_b ($r = -0.526$, $P =$
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846 338 0.036) in the pre-HA HST. The reduction in end-exercise T_b following HA was correlated
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848 339 with end-exercise T_{re} ($r = -0.638$, $p = 0.006$), T_{sk} ($r = -0.527$, $P = 0.030$), T_b ($r = -0.646$, $P =$
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850
851 340 0.005) and the within session ΔT_{re} ($r = -0.660$, $P = 0.004$), ΔT_{sk} ($r = -0.573$, $P = 0.020$) and
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853 341 ΔT_b ($r = -0.706$, $P = 0.002$) in the pre-HA HST. The reduction in the within session ΔT_b
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855 342 following HA was correlated with the end-exercise T_{sk} ($r = -0.679$, $P = 0.004$) and T_b ($r = -$
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857 343 0.600 , $P = 0.014$) and the ΔT_{re} ($r = -0.514$, $P = 0.042$), ΔT_{sk} ($r = -0.827$, $P < 0.001$), ΔT_b ($r =$
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859 344 -0.697 , $P = 0.003$), in the pre-HA HST. The increase in WBSR following HA was correlated
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861 345 with the average exercise T_{sk} in the pre-HA HST ($r = -0.565$, $P = 0.018$), whereas the decrease
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863 346 in average exercise heart rate following HA was correlated with the average ($r = -0.713$, $P =$
864
865 347 0.002) and end-exercise ($r = -0.757$, $P = 0.001$) heart rate in the pre-HA HST. The increase in
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867 348 blood volume following HA was not related to any of the variables measured in the pre-HA
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870 349 HST.

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874 351 To examine the specificity of the adaptive response *i.e.* whether those having a pronounced, or
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876 352 more limited, response for one adaption index also demonstrated a similar response for other
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878 353 indices of HA, correlation analysis were performed between the thermal indices of adaption
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880 354 (end-exercise T_{re} and T_b , within HST ΔT_{re} and ΔT_b) and the thermoregulatory (WBSR),
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888 355 haematological (Δ blood volume) and cardiovascular (average exercise heart rate) indices of
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890 356 adaption. This analysis indicated that the magnitude of increase in WBSR following HA was
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892 357 moderately related to the magnitude of the reduction in the within HST ΔT_{re} ($r = 0.487$, $P =$
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894 358 0.048), but there were no other significant relationships between the indices of adaption.
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897 359
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899 360 **Our analysis of the factors influencing the thermo-physiological responses to the initial HST**
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901 361 **(figure 2 a-d) demonstrated that the absolute H_{prod} (596(56) W, range 509:738 W) was strongly**
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903 362 **correlated with the within HST ΔT_{re} ($r = 0.609$, $P = 0.009$) and moderately correlated with the**
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905 363 **within HST session ΔT_{\square_b} ($r = 0.523$, $P = 0.038$) and WBSR ($r = 0.525$, $P = 0.030$). The relative**
906
907 364 **H_{prod} ($8.1(0.9) W \cdot kg^{-1}$, range $6.7:10.2 W \cdot kg^{-1}$) was moderately correlated with the end exercise**
908
909 365 **T_{re} ($r = 0.508$, $P = 0.037$) and the within HST ΔT_{re} ($r = 0.584$, $P = 0.014$). E_{req} ($r = 0.685$, $P =$
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911 366 0.002) and E_{req}/E_{max} ($r = 0.669$, $P = 0.003$) were strongly correlated with WBSR. Thereafter,**
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913 367 **we investigated whether those variables identified as being significantly related to our indices**
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915 368 **of acclimation in the initial HST were also related to the subsequent magnitude of adaptive**
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917 369 **response for that parameter. This analysis demonstrated a moderate negative correlation**
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919 370 **between the reduction in the within session ΔT_{re} following the HA **intervention** and the absolute**
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921 371 **H_{prod} in the initial HST ($r = -0.514$, $P = 0.035$), but there were no further significant correlations.**
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924 373 **4.0 DISCUSSION**

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927 374 Our findings demonstrate that the individual-variation in the adaptive responses to the 10-day
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929 375 HA **intervention** was not related to baseline VO_{2max} , previous exposure to a HA **intervention**,
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931 376 or the thermal ‘dose’ accrued during the HA **intervention**. In addition, there was limited
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933 377 evidence for strong relationships between the various indices of acclimation, indicating that the
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935 378 characterising of individuals as ‘high’, or ‘low’, responders to HA should be done so with
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937 379 reference to specific indices of HA, rather than as a ‘global’ classification. Importantly, some
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947 380 of the thermo-physiological responses during the initial HST were related to the magnitude of
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949 381 subsequent adaptive responses to the HA **intervention**, which suggests that some of these
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951 382 baseline responses may be useful in estimating the potential benefits that an individual may
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953 383 obtain from HA. However, we urge some caution, because the T_{re} and WBSR responses during
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955 384 the initial HST were also related to the inter-**participant** differences in H_{prod} and E_{req} , indicating
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957 385 that the design of the HST may also influence some of these initial thermo-physiological
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959 386 responses. Moreover, the inter-**participant** differences in H_{prod} during the initial HST were also
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961 387 related to the reduction in the within session ΔT_{re} following HA, indicating that the design of
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963 388 the HST may have influenced the scope for demonstrating adaption subsequently.
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969 390 It has often been suggested that the adaptive response to heat is augmented in those with a high
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971 391 **VO_{2max}** (e.g. Armstrong and Maresh, 1991; Casadio *et al.*, 2017), although closer inspection of
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973 392 the extant literature suggests that this assertion is based on a limited number of observations
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975 393 (Pandolf *et al.*, 1977). Likewise, it has been proposed that individuals with a high **VO_{2max}** are
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977 394 partially heat acclimated compared to those with lower **VO_{2max}** (e.g. Aoyagi *et al.*, 1997;
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979 395 Shvartz *et al.*, 1977). However, the present study has shown that baseline **VO_{2max}** (absolute or
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981 396 relative) was not related to the initial thermo-physiological responses to exercise in the heat,
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983 397 nor to the magnitude of the adaptive responses following the HA **intervention**. The reasons for
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985 398 these discrepant findings are unclear, although at the genomic level, transcriptome profile data
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987 399 from rodent models has shown that heat and exercise each induce specific transcriptional
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989 400 programmes (Kodesh *et al.*, 2011). Alternatively because, both the baseline **VO_{2max}** and the
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991 401 adaptive response of **VO_{2max}** to training have a considerable genetic component (Bouchard *et*
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993 402 *al.*, 2011a,b) the use of **VO_{2max}** as surrogate of training level and by extension the extent to
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995 403 which elevated thermal strain is encountered through habitual training will, at best, provide a
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997 404 crude estimate. **Future studies investigating this topic should consider analyses of in-depth**
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405 training data, rather than relying on measurement of VO_{2max} as a surrogate of training level.

406 Importantly, our observations have practical relevance; irrespective of baseline VO_{2max} ,
407 individuals required to exercise in high ambient temperatures should consider undertaking a
408 HA intervention and those with a high VO_{2max} should not consider themselves partially heat-
409 acclimated. Indeed, the belief that a VO_{2max} confers some HA may, in part, explain the recent
410 report that only 15% of athletes at the 2015 Athletics world Championship employed an HA
411 programme prior to competition (Périard *et al.*, 2017).

412
413 We also hypothesised that individuals who had undergone prior HA might demonstrate an
414 augmented acclimatory response. However, our analyses indicated that there were no
415 significant differences in the adaptive response of those individuals who had undertaken a prior
416 HA intervention. This finding is somewhat at odds with data showing that the magnitude of
417 some aspects of the acclimation response are increased with re-acclimation (Saat *et al.*, 2005).
418 Indeed, a recent meta-analysis concluded that the process of re-acclimation to heat was faster
419 than the initial acclimation, at least in terms of reduction in deep body temperature and
420 cardiovascular adaptations (Daanen *et al.*, 2018). Likewise, data from rodent studies has
421 demonstrated the presence of a cellular cytoprotective acclimation memory (Horowitz, 2016),
422 although the relevance of these observations for the whole-organism acclimation response is
423 not yet clear. Indeed, we were not able to measure aspects of cellular tolerance in the present
424 study and so cannot draw comparisons with Horowitz *et al.* (2016), whilst closer inspection of
425 the meta-analytic data indicates that in many of the primary studies the re-acclimation process
426 took place after a relatively short decay (*e.g.* Saat *et al.*, 2005) and some of the effects are
427 likely due to a baseline influence caused by retention of some of the initial adaptation to HA.
428 Importantly, our data indicate that the baseline HST responses of those who had undergone

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429 prior HA were not different from those who were undertaking HA for the first time suggesting
430 that the elapsed period between the acclimation was sufficient to enable full decay.

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432 In the present study we used a controlled hyperthermia HA **intervention** in which work rate
433 was adjusted in order to maintain a target T_{re} of 38.5°C-38.7°C on each day. In contrast to
434 traditional approaches, which typically use the same daily work-rate (*e.g.* Lind and Bass 1963;
435 Pandolf *et al.*, 1977; Senay *et al.*, 1976) and may, therefore, result in a diminishing thermal
436 forcing-function over the HA **intervention**, this approach maintains the thermal forcing-
437 function. Whilst our data indicate that the thermal ‘dose’ was well maintained over the course
438 of the HA **intervention** (no time effect for time $T_{re} > 38.5^\circ\text{C}$) there were notable inter-
439 **participant** differences in the total time accumulated above this thermal threshold. Previous
440 research suggests that the magnitude of adaptive response during HA is diminished when T_{re}
441 is $< 38.5^\circ\text{C}$ (Fox *et al.*, 1963), but there is no additional benefit when T_{re} is raised to 39.0°C
442 (Gibson *et al.*, 2015). Likewise, Lind and Bass (1963) demonstrate that the adaptive response
443 with 1×100 min daily exercise-heat exposure was greater than 2×50 minute daily exercise-
444 heat exposures, which they attributed to the greater amount of time spent elevating tissue
445 temperature with the multiple exposure protocol, whereas Fox *et al.*, (1963) demonstrated that
446 the adaptive response was greatest in individuals spending the most time with a T_{re} of $\sim 38.5^\circ\text{C}$.
447 In contrast, our data indicate that the indices of acclimation were not related to the time spent
448 with a $T_{re} > 38.5^\circ\text{C}$. The reason for this apparently discrepant finding is not clear. However, T_{re}
449 may not be the most appropriate index of thermal strain and \bar{T}_b might represent a better index
450 because it incorporates a measure of central and peripheral tissue temperature, which is
451 important for HA (Regan *et al.*, 1996), whereas the thermoeffector stimulus may be more
452 closely related to other parameters, such as E_{req} (Gagnon *et al.*, 2013).

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454 Inter-individual variability in the adaptive response to heat has led to the suggestion that
455 individuals might be classified as ‘responders’ or ‘non-responders’ to heat (Racinais *et al.*,
456 2012), as is the case with adaption to other stressors such as altitude (Chapman *et al.*, 1998),
457 or exercise (Bouchard *et al.*, 2011b). However, in some instances this classification has been
458 based upon a single reference parameter, such as plasma volume expansion (Racinais *et al.*,
459 2012) and when a range of adaptation indices are presented it is unclear whether the response
460 profile is consistent across indices (Racinais *et al.*, 2014). A moderate correlation was
461 demonstrated between the increase in WBSR and the magnitude of the reduction in the within
462 HST ΔT_{re} , however, on the whole, the various aspects of the adaptive response were not
463 correlated. Thus, the adaptive response to heat not only varies between individuals, but also
464 between indices of adaptation. For example, the magnitude of blood volume expansion was not
465 related to the changes in thermal indices of adaptation, WBSR, or exercise heart rate, thus an
466 individual who demonstrates a high adaptive response for blood volume may demonstrate a
467 low adaptive response for sudomotor, cardiovascular or thermal aspects of adaption. The basis
468 for this between-indices variation is unknown, although independence between aspects of the
469 adaptive response to heat has been demonstrated previously (Garrett *et al.*, 2009; Neal *et al.*,
470 2016a; Périard *et al.*, 2015). Baseline differences might contribute to some of the variation.
471 For instance, some individuals have a naturally-occurring high blood volume (Martino *et al.*,
472 2002), which might limit the scope for hypervolemia, with less influence on other indices of
473 adaptations. Alternatively, a low response for a given parameter may simply be a consequence
474 of an insufficient stimulus for adaption for that parameter; recent studies examining
475 heterogeneity in the training response have demonstrated that ‘non-responders’ to a standard
476 exercise training programme demonstrate a training response when the exercise ‘dose’ in
477 increased (Montero and Lundby, 2017).

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1183 479 Some of the physiological responses during the initial HST were related to the magnitude of
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1185 480 subsequent adaptive response. For instance, the ΔT_{\square_b} and $\Delta T_{\square_{sk}}$ recorded in the pre-HA HST
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1187 481 was related to the reduction in end-exercise T_{\square_b} , and the reduction in the within HST ΔT_{re} and
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1189 482 ΔT_{\square_b} following the HA **intervention**. Thus, a large increase in $T_{\square_{sk}}$ or T_{\square_b} during a standard
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1191 483 HST might be a useful index for assessing baseline HA status and in identifying those
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1193 484 individuals who will most benefit from HA. Likewise, the reduction in end exercise T_{re} and
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1195 485 T_{\square_b} over the HA **intervention** was related to the end-exercise T_{re} in the pre-HA HST,
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1197 486 indicating that individuals with the greatest end exercise T_{re} in the first HST also had the
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1199 487 greatest reduction in end-exercise T_{re} in T_{\square_b} after HA **intervention**. However, we urge some
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1201 488 caution with interpretation of this data. We hypothesised that the highest T_{re} and WBSR in the
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1203 489 initial HST would be observed in individuals with the highest H_{prod} and E_{req} , respectively, and
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1205 490 that these high baseline responses would provide greater scope for evidencing adaptation.
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1207 491 Indeed, our analysis confirmed that H_{prod} (in W or $W \cdot kg^{-1}$) was related to the T_{re} and T_{\square_b}
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1209 492 responses during the first HST, whereas the largest amount of variance in WBSR was explained
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1211 493 by E_{req} and E_{req}/E_{max} . These finding are consistent with the Cramer and Jay (2015) and indicate
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1213 494 that some of the response to the initial HST is determined by protocol design, rather than basal
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1215 495 HA state. Whilst this was not unexpected, most studies of HA do not attempt to standardise
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1217 496 heat production during the HST (*e.g.* Garrett *et al.*, 2009; Gibson *et al.*, 2015; Neal *et al.*,
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1219 497 2016ab; Pandolf *et al.*, 1977; Rendell *et al.*, 2017; Senay *et al.*, 1976; Shvartz *et al.*, 1977)
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1221 498 because it is typically assumed to be of little relevance for within-participants design so long
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1223 499 as the same external work-rates are used post-HA. However, our analysis also demonstrated a
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1225 500 moderate negative correlation between the reduction in the within HST ΔT_{re} following HA and
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1227 501 the absolute H_{prod} (W) in the initial HST, suggesting that the design of the initial HST may also
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1229 502 affect the subsequent response. Although we acknowledge that correlation is not evidence of
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1231 503 causality, we propose that this represents a potential baseline effect whereby those
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1242 504 demonstrating low-baseline HST response (due to the low H_{prod}) have less scope for evidencing
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1244 505 an adaptive response. The precise design of any HST will depend upon the nature of the
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1246 506 research question. However, future studies examining the variability in the adaptive response
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1248 507 to HA should consider standardisation of H_{prod} when assessing basal acclimation status and the
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1250 508 subsequent adaptive responses to a HA programme, particularly when there are differences in
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1252 509 participant $VO_{2\text{max}}$. Importantly, none of the other adaptation indices were related to the HST
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1254 510 design and the majority of the variance in the HA response remains unaccounted for;
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1256 511 subsequent studies should examine the influence of genetic and epigenetic factors on the
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1258 512 variability in the HA response.
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1263 514 **5.0 Conclusion**

1265 515 At the cohort-level, there was clear evidence of HA following the 10-day HA intervention, but
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1267 516 pronounced variation was evident at the individual-level. This inter-participant variation was
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1269 517 not related to factors that have putatively been proposed to influence the adaptive response to
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1271 518 heat, including $VO_{2\text{max}}$, a history of prior HA, and the thermal ‘dose’ accrued during the HA
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1273 519 intervention. The magnitude of adaptive response is, in the main, specific to the index of
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1275 520 adaption; individuals who demonstrate a high, or low, adaptive response on one index of HA
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1277 521 do not automatically demonstrate a similar response across the spectrum of HA indices. Some
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1279 522 of the thermo-physiological responses during the initial HST were related to the magnitude of
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1281 523 subsequent adaptive response, indicating that the initial response to a standard HST may have
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1283 524 utility in identifying those individuals who will obtain the greatest adaptations from HA.
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1285 525 However, some of the initial thermo-physiological responses may also have been influenced
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1287 526 by the design of the HST; ΔT_{re} was strongly related to H_{prod} and WBSR was strongly related to
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1289 527 E_{req} . Moreover, the reduction in the within session ΔT_{re} following HA was related to the H_{prod}
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1291 528 in the initial HST, indicating that the design of the HST may also have influenced the scope
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529 for demonstrating adaption. Nevertheless, the substantial majority of the inter-individual
530 variance in the adaptive response to heat remains unaccounted for and future studies should
531 seek to increase understanding of the factors contributing to this variance.

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537
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542 **8.0 DECLARATION OF INTERESTS**

543 The authors have no conflicts of Interest to Declare

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545 **9.0 REFERENCES**

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696 **10 FIGURE CAPTIONS**

697 **Figure 1.** a) relationship between pre heat acclimation (HA) haemoglobin concentration and
698 the reduction in end-exercise T_{re} following HA; b) relationship between ΔT_{sk} in the pre HA
699 Heat Stress Test (HST) and the reduction in the within HST ΔT_{re} following HA; c) relationship
700 between ΔT_b in the pre HA HST and the reduction in the end-exercise T_b following HA; d)
701 relationship between ΔT_{sk} in the pre HA HST and the reduction in the within HST ΔT_b
702 following HA; e) relationship between average exercise T_{sk} in the pre HA HST and the
703 increase in whole body sweat rate following HA; f) relationship between end-exercise heart
704 rate in the pre HA HST and the reduction in the average exercise heart rate following HA.

705
706 **Figure 2.** Correlation coefficients for associations between thermoregulatory responses during
707 the initial heat stress test and relevant independent variables: a) end exercise T_{re} ($^{\circ}\text{C}$) = light
708 grey bars, ΔT_{re} ($^{\circ}\text{C}$) = dark grey bars); b) end-exercise T_b ($^{\circ}\text{C}$) = light grey bars), ΔT_b ($^{\circ}\text{C}$)
709 = dark grey bars; c) whole body sweat rate ($\text{L}\cdot\text{hr}^{-1}$); d) average exercise heart rate ($\text{beats}\cdot\text{min}^{-1}$). * $P < 0.05$; ** $P < 0.01$. BSA = body surface area; $\text{VO}_{2\text{max}}$ = maximum rate of oxygen uptake;
710 H_{prod} = heat production; E_{req} = evaporative requirement for heat balance; E_{max} = maximum rate
711 of evaporation to the environment; ND = no denomination.

714 **11.0 AUTHOR BIOGRAPHIES**



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716 Dr Jo Corbett is an Associate Head in the Department of Sport and Exercise Science at the
717 University of Portsmouth, UK. He is a member of the Extreme Environments Laboratory,
718 where his research examines the effect of environmental stressors, alone and in combination,
719 on human performance and health.

720



721

722 Dr Rebecca Rendell (née Neal) completed her Ph.D. at the University of Portsmouth in 2017
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724 London in 2013, and undergraduate degree in Sport and Exercise Science from the University
725 of Birmingham in 2012. Rebecca is now a Lecturer in Exercise Physiology at Bournemouth
726 University and conducts research in the areas of exercise and extreme environmental
727 physiology and sports performance.

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731 Dr Heather Massey is a senior lecturer in Sport Exercise and Health. Her research interests
732 focus on human physiology in extreme environments. Primarily studying the effect
733 combinations of environmental stressors (cross-adaptation), such as exposure to the cold and
734 hypoxia, have on human thermoregulatory, vascular, respiratory and autonomic function.

735



736
737 Dr Joseph Costello is a Senior Lecturer in exercise physiology and a member of the Extreme
738 Environments Laboratory at the University of Portsmouth, UK. His research interests are
739 directed towards i) understanding the physiological effects of various stressors (e.g. exercise,
740 extreme environments, clothing) on human performance and ii) establishing evidence-based
741 practice in sport and exercise science through the publication of high quality systematic reviews
742 and meta-analyses.

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745 Prof Mike Tipton is a professor of human & applied physiology with an interest in the
746 physiological and pathophysiological response to extreme environments, and the selection,
747 preparation and protection of those entering such environments

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